Methods for Identifying Causes of Toxicity in Whole-Sediments

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A thesis submitted in fulfilment of the degree of Doctor of Philosophy

2009

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ACKNOWLEDGEMENTS

My journey over the past four years wouldn’t have been possible without the guidance, support and encouragement from selected individuals who have inspired me to continuously challenge myself.

Foremost, I express my gratitude and admiration to my supervisor Dr Stuart Simpson (CSIRO). Thank you for your wisdom, fundamental knowledge and guidance that you have imparted on me. You have provided me with honest and critical reviews of my work which I can’t show enough appreciation for. Appreciation is expressed to my university supervisor Professor Bill Maher (University of Canberra). Your distant support and assistance with my research has been invaluable. Thank you to Dr Graeme Batley (CSIRO). Your incredibly swift and precise editing and insightfulness has been immensely appreciated and admired.

This research was supported by an Industrial Postgraduate Research Award and CSIRO PhD scholarship, and was undertaken at CSIRO Land and Water (Lucas Heights), whom I appreciatively acknowledge for the supply of materials, equipment, technical assistance and vast knowledge. Thank you to my project sponsors; Rio Tinto, BHP Billiton, Xstrata, without their funding this project would not have been possible. I would also like to extend my appreciation to Dr Ruth Eriksen (University of Tasmania) for supply of sediments.

Thank you to CSIRO staff for their technical assistance in the lab and advice. Merrin Adams & Monique Binet for your endless supply of algae, use of the flow cytometer and wealth of knowledge of algae, and Dr Anthony Chariton for providing me with decisive statistical advice. I would like to specially thank David Spadaro for your support and assistance in the sediment lab, and importantly your genuine friendship. You made 2J a truly an enjoyable place to work.

Many thanks to my fellow PhD students for your friendship, encouragement and support over the years, you have helped in making this journey a memorable one. I wish you all the very best in your future endeavours. Thanks to my friends for your support, encouragement and motivation throughout the past four years. I also wish to acknowledge the support of my colleagues at ESA, especially Dr Rick Krassoi and Chris Doyle for hiring a poor PhD student and supporting a flexible work schedule during the final weeks on my PhD.

Finally, and most importantly my family to whom I cannot convey enough appreciation to. I wish to express my deepest gratitude to my parents, Rosa and Dragi, brother Oliver for there unconditional love, support and endless encouragement (and lets not forget the sometimes temperamental behaviour). To my sister Sonja, I thank you the most for being a constant source of encouragement, motivation, support, love and positive distraction. You have provided me with support and affection through the most testing of times over the past years. I am also extremely grateful for the late nights you dedicated away from Alan and Oscar to help format and edit my thesis. I am indebted to you.

And last, but not forgotten, thank you to Jordan and Oscar, who have both been a positive distraction, and have given me silent affection.

LIST OF PUBLICATIONS AND CONFERENCE PROCEEDINGS

Pubblications

Simpson, S.L., **Micevska, T**., Adams, M.S., Stone, A. and Maher, W.A. (2007). Establishing the cause-effect relationships in hydrocarbon-contaminated sediments using the sublethal response of the benthic marine alga *Entomoneis* cf *punctulata*. *Environ Toxicol Chem* **26**,163-170.

Micevska, T. and Simpson, S.L. (2008). Modifying TIE methods to demonstrate dietary toxicity in whole-sediment toxicity tests. *Integ Environ Assess Manag* **4**,369-370.

Spadaro, D.A., **Micevska, T**. and Simpson, S.L. (2008). Effect of nutrition on toxicity of contaminants to the epibenthic amphipod *Melita plumulosa*. *Arch Environ Contam Toxicol* **55**, 593-602.

Conference Presentations

Micevska, T., Simpson, S.L. and Maher, W.A. (2007). Use of TIE concepts to identify the dietary toxicity of metals in marine sediments. Conference proceeding. SETAC, November 7-11, Milwaukee, USA.

Micevska, T., Simpson, S.L. and Maher, W.A. (2008). Modifying TIE methods to demonstrate dietary toxicity in whole-sediment toxicity tests. Conference proceeding. SETAC, August 3-7, Sydney, Australia.

Micevska, T., Simpson, S.L. and Maher, W.A. (2006). Determining the contribution of dissolved and particulate metal exposure pathways to the toxicity of marine sediments. Conference proceeding. RACI, September 24-28, Perth, Australia.

Micevska, T., Simpson, S.L. and Maher, W.A. (2005). Development of Toxicity Identification Evaluation Procedures for Contaminated Whole Sediments. ASE, September 25-28, Melbourne, Australia.

Micevska, T., Simpson, S.L. and Maher, W.A. (2005). Identifying causes of toxicity in sediments containing mixtures of toxicants. Conference proceeding. RACI R&D Topics Meeting. December 10-13, Mornington Peninsula, Australia.

ABSTRACT

Whole-sediment toxicity identification and evaluation (WS-TIE) is a relatively new approach for assessing the cause of toxic effects to benthic organisms in sediments. Akin to aqueous TIE methods, the premise of the WS-TIE method is that the chemical toxicant(s) responsible for observed effects can be identified through a series of treatments that are designed to reduce the bioavailability and, thus, toxicity of key contaminant classes. While standardised WS-TIE methods have been developed for a range of contaminants (US EPA, 2007), many contaminated sediments exist for which the methods can not adequately identify the cause(s) of toxicity. Standard WS-TIE methods primarily manipulate the toxicity of dissolved contaminants, but do not address effects that may occur via dietary exposure to chemical contaminants.

The research presented herein, recognises that standard WS-TIE methods do not address all of the major contaminant exposure pathways for some benthic organisms. New WS-TIE methods to address toxic effects of those contaminants acting via dietary exposure routes were developed as a part of this research. The new methods were specifically designed for whole-sediment toxicity tests using the epibenthic amphipod *M. plumulosa;* a deposit feeding species that can display acute toxicity from dietary exposure to contaminated sediments (Simpson and King, 2005; Mann and Hyne, 2008; Spadaro *et al*., 2008). WS-TIE methods using the microalgae *Entomoneis* cf *punctulata* were also developed to compliment results achieved using *Melita plumulosa* in sediment quality assessments.

New WS-TIE treatments were developed to modify the organism's exposure to sediment contaminants by modifying the bioaccessibility of particulate-associated contaminants (PACs) to *M. plumulosa*. The two new techniques were principally employed to achieve this goal. Firstly, a mesh exposure chamber (MEC) was developed that effectively prevented *M. plumulosa* from ingesting sediments, but did not modify the exposure to dissolved contaminants in the overlying water. Secondly, resins deployed at the sediment surface, metal chelating resin-top (MCR-Top) or carbonaceous adsorbent resin-top (CAR-Top) to both remove from the dissolved phase, metals and organic contaminants, respectively. It was demonstrated that sediment nutrition had a large influence on the outcome of whole-sediments toxicity tests, and a food addition (FOOD) treatment was incorporated into the suite of WS-TIE treatments to help differentiate between the natural effects caused by nutritionally-poor sediments and the toxic effects of dietary exposure to contaminants.

For the amphipod *M. plumulosa*, the standard WS-TIE methods were demonstrated to be ineffective for reducing, or eliminating, the toxicity of a range of sediments. For ~80% of the sediments investigated, >50% of the toxicity to *M. plumulosa* was un-accounted for using standard WS-TIE methods. By applying the new WS-TIE treatments in combination with the standard WS-TIE methods, >90% of toxicity could be identified for each of the sediments. For almost half these sediments, PACs were considered to have significantly contributed to toxicity (50-100%). Additionally, new WS-TIE treatments were able to improve the identification of dissolved metals and hydrophobic organic contaminants (HOCs), when compared to standard WS-TIE methods alone. The application of both standard and new WS-TIE methods identified dissolved metals and HOCs as the chief causes of toxicity to *M. plumulosa* in ten sediments containing a wide range of contaminants.

Apparent toxicity due to inadequate sediment nutrition was demonstrated to confound the interpretation of the WS-TIE studies. It was found to be beneficial to apply a 'minimal' feeding regime, comprising approximately 0.06 mg fish-food per amphipod on days 3 and 7 of 10-d toxicity tests, to ensure that a lack of nutrition did not cause toxic effects in sediments containing low nutritional value (e.g. sandy sediments with low amount of organic matter). Sediments that contained ≥10% fine particles (silt, <63 μm) and >2% organic carbon were determined to have adequate nutrition and FOOD treatments were unnecessary. The addition of the FOOD treatment in WS-TIEs was generally a useful tool for discerning poor nutrition from toxicity to *M. plumulosa*. However, in applying a FOOD treatment to WS-TIEs, it was observed that selective feeding could mask the toxic effects of some contaminants, namely hydrophobic organic chemicals. Therefore, the FOOD treatment was used cautiously to discern poor nutrition from toxicity using additional lines-of evidence, such as knowledge of changes to the organism's sensitivity to contaminants and contaminant concentration-effect relationships.

For the microalgae, *E.* cf *punctulata*, for which the only significant contaminant exposure pathway is believed to be the passive diffusion of dissolved contaminants or their free ions across the cell surface, a rapid WS-TIE method based on FDA fluorescence inhibition in *E.* cf *punctulata* using flow cytometry was developed for dissolved metals (metal chelating resin, MCR^x, treatment), organic contaminants (carbonacsous adsorbent resin, CAR, treatment), and ammonia (zeolite, ZEO, treatment). These treatments were effective for reducing the toxicity of contaminants in both natural -contaminated and spiked-sediments.

The identification of all the chemical toxicants within contaminated sediments was generally found to be very difficult. The variability between replicates was usually high, ranging from 0-19% (amphipod survival). The use of a multiple lines-of-evidence approach with WS-TIE is recommended for sediments containing a wide variety of chemical contaminants. The lines-of-evidence used to enhance the understanding of toxicants in the sediments of this study included: (i) chemical analyses of the test waters, (ii) analyses of physical/chemical properties of the sediment (i.e. total particulate metals and acid extractable metals, organic contaminants, sediment fractionation, total organic carbon, etc), (iii) understanding of the organisms contaminant-exposure pathways, (iv) quantifying the role of nutrition to sediment toxicity (i.e. silt and TOC content), (v) measuring the effects of added food on organism sensitivity, contaminant bioavailability, feeding behaviour or physiology (i.e. depuration and detoxification processes), and (vi) understanding the physiology and behaviour of the organism, and the factors that may have significant effects.

In order to strengthen the lines-of-evidence for interpreting WS-TIE data, contaminant concentration-effect relationships were determined for contaminants often observed in sediments. For *M plumulosa*, the 10-d EC_{50} for dissolved un-ionised ammonia, copper, and zinc were 980, 75, and 220 µg/L, respectively. The IC_{50} values for dissolved copper and zinc to *E*. cf *punctulata* were 13 µg/L and 1,500 µg/L, respectively. The IC_x and EC_x values were used to determine the specific contaminant(s) causing toxicity and/or the contribution of these contaminants to observed toxic effects in sediments containing a mixture of contaminants.

The effect of contaminants on the growth of *M. plumulosa*, and the ability of WS-TIE methods to determine which stressors were the causes of any growth effects, was assessed. Results from the natural-contaminated sediments and the chemical-spiked sediments demonstrated that the growth, measured as amphipod size (area), was a more sensitive indicator of toxicity than acute survival. However, WS-TIE methods were far less effective for identifying toxicity using amphipod growth than survival. Difficulties with applying a growth toxicity indicator using *M. plumulosa* arose from the significant influence of sediments physico-chemical properties, storage and handling of sediment and feeding regime during whole-sediment toxicity tests.

New WS-TIE methods and additional lines-of-evidence described in this research provide a more comprehensive approach for sediment quality assessment, specifically for those organisms exposed to sediment-associated contaminants via their diet. However, further research is required to enhance techniques for delineating toxicity due to dissolved and particulate-associated toxicity, and identifying specific classes of PACs. Due to the variability associated with whole-sediment toxicity tests, the use of multiple lines-of-evidence is essential for WS-TIE procedures.

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AEM: acid extractable metals

CAR: carbonaceous adsorbant resin

CC: coconut charcoal

EDTA: ethylenediaminetetraacetic acid

FDA: flouroscein diacetate

GCC: granular coconut charcoal

HOC: hydrophobic organic contaminant

ISQG: interim sediment quality guidelines

LOEC: lowest observed effect concentration

MCR: metals chelating resin

MEC: mesh exposure chambers

NOEC: no observed effect concentration

PAC: particulate-associated contaminants

PAH: polycyclic aromatic hydrocarbon

PCC: powdered coconut charcoal

TBT: tributyl tin

TIE: toxicity odentification evaluation

TOC: total organic carbon

TPM: total particulate metals

WS-TIE: whole-sediment toxicity identification evaluation

ZEO: zeolite